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EXAMINER	
HILL, KEVIN KAI	

ART UNIT	PAPER NUMBER
1633	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/815,262	ENGELHARDT ET AL.	
	Examiner	Art Unit	
	KEVIN K. HILL	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 May 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,2,5-7,9-25,27,29-32,43,44,46,48-51,53,55-59,62 and 65* is/are pending in the application.

4a) Of the above claim(s) *25,27,29-32,51,53 and 55-59* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2,5-7,9-24,43,44,46,48-50,62 and 65* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *May. 6, 2009*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

Election/Restrictions

Applicant has elected with traverse the invention of Group I, Claims 1-32 and 43-60, drawn to a method of enhancing recombinant adeno-associated virus (rAAV) transduction in mammalian cells, comprising contacting the mammalian cells with at least one agent in an amount effective to additively or synergistically enhance rAAV transduction.

Within Group I, Applicant has further elected the restricted subgroup "A", wherein the at least two agents additively enhance rAAV transduction.

Within Group I, Applicant has elected the following species:

i) the biological functionality species "doxil" and "LLnL", as recited in Claims 21 and 60. However, upon further examination of the subject matter, the Examiner has extended the species under examination to include doxorubicin.

ii) the cell type species "mammalian lung cell", as recited in Claims 16 and 48.

iii) the polypeptide biological functionality species "cystic fibrosis transmembrane conductance regulator (CFTR)", as recited in Claim 20, wherein CFTR is found in both rAAVs.

Amendments

Applicant's response and amendments, filed May 4, 2009, to the prior Office Action is acknowledged. Applicant has cancelled Claims 3-4, 8, 26, 28, 33-42, 45-47, 52, 54, 60-61 and 63-64, withdrawn Claims 25, 27, 29-32, 51, 53 and 55-59, amended Claims 1 and 43, and added new claims, Claim 65.

Claims 25, 27, 29-32, 51, 53 and 55-59 are pending but withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a non-elected invention, there being no allowable generic or linking claim.

Claims 1-2, 5-7, 9-24, 43-44, 46, 48-50, 62 and 65 are under consideration.

This application contains claims, Claims 25, 27, 29-32, 51, 53 and 55-59, drawn to an invention nonelected with traverse in the reply filed on March 19, 2007, wherein the restriction/election requirement was made FINAL in the Office Action mailed May 15, 2007. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP §821.01.

Examiner's Note

Unless otherwise indicated, previous objections/rejections that have been rendered moot in view of the amendment will not be reiterated. The arguments in the May 4, 2009 response will be addressed to the extent that they apply to current rejection(s).

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Priority

Applicant's claim for the benefit of a prior-filed application parent provisional applications 60/459,323, filed on March 31, 2003 and 60/512,347, filed on October 16, 2003 under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged.

Information Disclosure Statement

Applicant has filed Information Disclosure Statements on May 6, 2009, that has been considered. The signed and initialed PTO Forms 1449 are mailed with this action.

Claim Objections

1. **The prior objection to Claims 43 and 61 is withdrawn** in light of Applicant's amendments to the claims.

Claim Rejections - 35 USC § 112

2. **The prior rejection of Claims 1-2, 5-7, 9-24, 43-44, 48-50 and 62 under 35 U.S.C. 112, first paragraph,** as failing to comply with the written description requirement **is withdrawn** in light of Applicant's amendment to Claims 1 and 43.

3. **The prior rejection of Claims 1-2, 4-7, 9-24, 43-44, 46, 48-50 and 61-64 under 35 U.S.C. 112, first paragraph**, as failing to comply with the enablement requirement **is withdrawn** in light of Applicant's amendment to Claims 1 and 43, and in preference for the rejection set forth below.

Applicant's amendments to the claims have necessitated the following new grounds of rejection under 35 U.S.C. 112, first paragraph.

4. **Claims 1-2, 5-7, 9-24, 43-44, 46, 48-50, 62 and 65 are rejected under 35 U.S.C. 112, first paragraph**, because the specification, while being enabling for an *in vitro* method to enhance recombinant adeno-associated (rAAV) transduction of a mammalian cell comprising contacting the mammalian cell with at least one rAAV and two different agents that each enhance intracellular rAAV transduction of a mammalian cell in an amount effective to more than additively enhance rAAV transduction, wherein the first agent is doxorubicin and the second agent is the tripeptide aldehyde N-acetyl-L-leucyl-L-leucyl-norleucinal (LLnL) or N-carbobenzoxyl- L-leucinyl-L-leucinyl-L-leucinal (Z-LLL) that inhibits proteosome proteolytic activity, does not reasonably provide enablement for an *in vitro*, *ex vivo* or *in vivo* method to enhance rAAV transduction of an enormous genus of mammalian cells, nor contacting the mammalian cell with a combination of an enormous genus of at least two or more agents in an amount effective to more than additively enhance rAAV transduction. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

While determining whether a specification is enabling, one considers whether the claimed invention provides sufficient guidance to make and use the claimed invention. If not, whether an artisan would have required undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets the enablement requirements, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue" (*In re Wands*, 858 F.2d 731, 737, 8 USPQ2ds 1400, 1404 (Fed. Cir. 1988)). Furthermore, USPTO does not have laboratory facilities to test if an invention will function as claimed when working examples are not disclosed in the specification. Therefore, enablement issues are raised and discussed based on the state of knowledge pertinent to an art at the time of the invention. And thus, skepticism raised in the enablement rejections are those raised in the art by artisans of expertise.

The Breadth of the Claims and The Nature of the Invention

The breadth of the claim is exceptionally large for encompassing methods of enhancing the transduction of a multitude of recombinant adeno-associated viruses (rAAV) of different serotypes, i.e. serotypes 1-11 and permutations thereof, having distinct tropisms to distinctly different mammalian cell types, e.g. muscle, epithelial, endothelial, neural, hematopoietic, hepatic, etc…, wherein the transduction may occur *in vitro*, *ex vivo* or *in vivo*, the method comprising the combination of two or more structurally and functionally diverse agents to enhance rAAV transduction in the target cell. Applicant broadly contemplates the term 'viral transduction' to include a broad genus of distinctly different and mutually exclusive cell biological processes, such as endocytosis, trafficking and processing of the rAAV through intracellular compartment(s), e.g., endosomal compartments, decreased viral nucleic acid or protein degradation, increased viral uncoating, or increased nuclear transport of virus or the viral genome, agents that interact with cytoskeletal elements, e.g., microtubules or microfilaments (pg 8, lines 23-27).

The inventive concept in the instant application is that *in vitro* rAAV2 and rAAV5 transduction of a mammalian host cell may be enhanced by contacting the cell with the proteosome inhibitor LLnL or Z-LLL in combination with the antibiotic/chemotherapeutic compound doxorubicin.

The State of the Prior Art, The Level of One of Ordinary Skill and The Level of Predictability in the Art

The level of one of ordinary skill in the art of recombinant adeno-associated viral vector design and delivery is considered to be high.

The prior art is silent with respect to the administration of agents, particularly the elected embodiments DOXIL® and LLnL, to enhance rAAV transduction. The claimed methods recite the administration of agents to alter distinctly different cell biological processes to enhance transduction. However, Goncalves (Virology J. 2: 43; 17 pages, 2005) teaches that the events and processes that regulate the trafficking of AAV particles into the nucleus are still not fully understood (pg 5 of 17). An increasingly important area in the development of AAV as a vector concerns the engineering of altered cell tropisms to narrow or broaden rAAV-mediated gene

delivery and to increase its efficiency in tissues refractory to AAV2 infection. Cells can be poorly transduced by prototype rAAV2 not only because of low receptor content but also owing to impaired intracellular virion trafficking and uncoating or single-to-double strand genome conversion. Thus, considering that these processes depend either directly or indirectly on capsid conformation, cell targeting strategies determine not only the cell type(s) with which the vector interacts but also critically affect the efficiency of the whole gene transfer process. (pg 7 of 17) Several of these approaches rely on the modification by chemical, immunological or genetic means of the AAV2 capsid structure endowing it with ligands that interact with specific cell surface molecules. Another route to alter rAAV tropism exploits the natural capsid diversity of newly isolated serotypes by packaging rAAV2 genomes into capsids derived from other human or non-human AAV isolates. To this end, up until now, most researchers employ hybrid *trans*-complementing constructs that encode *rep* from AAV2 whereas *cap* is derived from the serotype displaying the cell tropism of choice. For example, experiments published recently using rAAV2 genomes pseudotyped with coats from AAV6 and AAV8 revealed stunning gene transfer efficiencies when these vectors were administered alone at high doses or in combination with a blood vessel permeating agent.

Duan et al (J. Clin. Invest. 105:1573-1587, 2000; *of record) teach that the administration of tripeptide protease inhibitors, e.g. LLnL, increase rAAV gene delivery *in vitro* (pg 1573, Abstract). However, this phenomenon is not universal in that the proteosome inhibitor did not affect transduction of skeletal or cardiac muscle, indicating that tissue-specific ubiquitination of viral capsid proteins interfere with rAAV-2 transduction.

Similarly, compounds such as etoposide, hydroxyurea, and campothecin were effective at enhancing rAAV transduction when utilized singularly but when used in combination produced no additive or synergistic effects (Specification, pg 6, lines 6-9, citing Russel et al., 1995).

Given the limited teachings in the art regarding the co-administration of two or more compounds designed to specifically alter particular cell biological processes to intentionally enhance rAAV transduction of an enormous genus of mammalian cell types *in vitro*, *ex vivo* and *in vivo*, one of ordinary skill in the art would reasonably conclude that a high degree of unpredictability regarding an *a priori* determination that any specific compound will enhance

viral transduction more than additively. It necessarily follows that the art recognizes significant unpredictability for any two agents to yield a more than additive [synergistic] interaction to enhance viral transduction.

Furthermore, Applicants declare (Englehardt and Yan Decl., filed May 6, 2009; see discussion below) that in the absence of data, it is not predictable what combinations of agents have any effect, let alone have an additive or synergistic effect on rAAV transduction (¶8). Compounds with similar mechanisms of action may, under some conditions, yield a positive, but not synergistic, effect (¶8). No predictable correlation is found between rAAV serotypes (rAAV-2 or rAAV-5) and enhanced transduction of a particular mammalian cell type (IB3, A549, HeLa) (¶11, Table 1). That is to say, a synergistic effect between a first and second agent in a first cell type with a first rAAV serotype cannot be predictably extrapolated to a second cell type and a second rAAV serotype. The effect of a first double agent combination (Dox and LLnL) does not predictably extrapolate to a second double agent combination (Velcade® and LLnL; Velcade® and Dox) (Table 1). It is not predictable what combinations of agents have any effect, let alone a positive or synergistic effect, on AAV transduction (¶13).

Applicant states that because of its cytotoxicity, the use of doxorubicin in a clinical setting is counter indicated due to its cumulative systemic toxicity. In addition, the use of a toxic agent, such as doxorubicin, may compromise cell viability, thereby defeating the goal of enhanced transduction of cells with rAAV encoding a therapeutic gene product. Given that doxorubicin binds DNA, it might be expected to inhibit rAAV transduction, as rAAV is present as a double-stranded DNA molecule in the nucleus (¶7).

The Existence of Working Examples and The Amount of Direction Provided by the Inventor

The specification discloses DOXIL® to be a chemotherapeutic agent (pg 79, line 20) and is disclosed to enhance rAAV2 transduction (pg 82, line 31). DOXIL® is the liposomal formulation of doxorubicin (pg 80, line 17) that is an approved antibiotic (pg 9, line 25) and chemotherapeutic agent (pg 79, line 20). The specification also discloses that the tripeptide aldehyde, "LLnL", is a proteosome inhibitor that can enhance transduction (pg 5, line 30), but acts at a point distal to (that is, after) virus binding and entry (pg 70, line 8). LLnL and doxorubicin synergistically enhance rAAV transduction *in vitro*, as measured by reporter gene

expression 1000-fold, while individually, doxorubicin and LLnL enhanced rAAV reporter gene expression 100- and 10-fold, respectively (pg 12, lines 8-10; Figure 13; Declaration, Table 1).

The specification discloses the use of an optimized dose combination (pg 100, line 22) of LLnL and Dox for enhancing rAAV2 transduction *in vitro* (Figure 13). For *in vivo* studies, it is necessary to use the tripeptide Z-LLL was employed in place of LLnL because solubility in ethanol is much higher for Z-LLL. LLnL and Z-LLL perform similarly to augment rAAV in human polarized airway epithelia (Duan et al., 2000). The *in vivo* administration of DOXIL®, the liposomal formulation of doxorubicin, resulting in the enhancement of rAAV2 transduction (Figures 2-3). The *in vivo* administration of DOXIL® in combination with the tripeptide aldehyde Z-LLL, wherein said combination fails to achieve more than additive enhancement of rAAV2 transduction until 6 weeks after nasal aspiration via a specific dosing regimen: 3 times with 10μl of $2x10^9$ particles per μl in 40μl, for a total of 6×10^{10} particles, and administration of Z-LLL at a concentration of 200μM and doxorubicin at a concentration of 200μM (Figure 9D). By three months post-infection, synergism was no longer observed (pg 86, lines 26-29).

However, the claims lack enablement for the full scope because while the specification discloses working examples *in vitro* cell culture (e.g. pg 78, Example 3), the specification fails to provide an predictable nexus between the agent concentration for use *in vitro* and the agent concentration for use *in vivo*. For example, the specification discloses that DOXIL® previously tested negative in cell line screening while the free compound doxorubicin tested positive in cell line screening (pg 82, lines 2-4), thereby evidencing that a result achieved *in vitro* does not predictably correlate with a result to be achieved *in vivo*. The specification fails to disclose the dosing of LLnL in combination with doxorubicin to achieve a more than additive effect of enhancing rAAV transduction *in vivo*.

Applicant's declaration clearly evidences that one of ordinary skill in the art cannot predictably extrapolate the dosing of a first agent combination and the dosing of a second agent combination. Compounds with similar mechanisms of action may, under some conditions, yield a positive, but not a synergistic, effect. Not all combinations of agents that individually enhance AAV transduction yield a positive or synergistic effect and that some combinations in fact

reduce AAV transduction efficiency (Remarks, pg 20, ¶2). Thus, the instant specification fails to provide the necessary nexus establishing predictability.

Furthermore, the specification provides only general guidelines regarding the drug formulation and dosing (pgs 52-59). For example, the specification discloses the agent may comprise 0.1 to 99.9% by weight of the pharmaceutical formulation (pg 52, lines 29-31). The specification discloses that different cell types require different amounts of the agent to achieve enhancement of rAAV transduction (pg 81, lines 15-18), and that there are striking differences in the kinetics and longevity of induction by Z-LLL between *in vivo* studies with rAAV-2 and rAAV-5. The results highlight that different agents and vectors achieve different results (pg 87, lines 5-6).

Applicant also declares that because of its cytotoxicity, the use of doxorubicin in a clinical setting is counter indicated due to its cumulative systemic toxicity (¶7). In addition, the use of a toxic agent, such as doxorubicin, may compromise cell viability, thereby defeating the goal of enhanced transduction of cells with rAAV encoding a therapeutic gene product. However, the specification discloses only the single administration of doxorubicin *in vivo*, and fails to disclose the dosage/dosing regimen to avoid the cumulative systemic toxicity so as to enhance rAAV transduction in the treatment of a chronic disease such as cystic fibrosis, which would likely require multiple administrations of a gene therapy vehicle, e.g. rAAV comprising the CFTR gene, as claimed.

The Quantity of Any Necessary Experimentation to Make or Use the Invention

Thus, the quantity of necessary experimentation to make or use the invention as claimed, based upon what is known in the art and what has been disclosed in the specification, will create an undue burden for a person of ordinary skill in the art to demonstrate that the administration of an enormous genus of structurally and functionally diverse compositions so as to affect a broad genus of distinctly different and mutually exclusive cell biological processes will yield an additive functional interaction so as to enhance rAAV transduction of enormous genus of mammalian cells (both organisms and physiological cell types), wherein the transduction may occur *in vitro*, *ex vivo* or *in vivo*.

The claims require undue experimentation because the artisans must determine for themselves:

i) the *in vivo* dosing and dosage regimen of an enormous genus of two or more agent combinations to administer to a host subject with no predictable expectation of success to find a combination that achieves a more than additive effect in enhancing rAAV transduction,

ii) the *in vitro* and/or *ex vivo* dosing of an enormous genus of two or more agent combinations to an enormous genus of mammalian cell types with no predictable expectation of success to find a combination that achieves a more than additive effect in enhancing rAAV transduction,

iii) the appropriate and unpredictable timepoint after the two or more agents are administered from which one is to assay for the more than additive effect *in vivo* as per a given tissue, e.g. at six weeks, but not two weeks, three weeks, etc…, in heart, lung, kidney, brain, etc..,

iii) the *in vivo* dosing and dosage regimen for each particular cell type, e.g. epithelial, endothelial, hematopoietic, neural, etc…, because the results achieved with a first cell type are not predictably extrapolated to the results one may expect to achieve with a second cell type, as evidenced by Applicant's own work (Declaration, Table 1),

iv) assaying each rAAV serotypes 1-11, and permutations thereof, for each two or more agent combinations for each cell type,

v) the *in vivo* dosing and dosage regimen to avoid cumulative systemic toxicity of the two or more agent(s), and

vi) whether or not the results of each two or more agent combinations that achieves a more than additive effect *in vitro* can be predictably extrapolated to usage *in vivo*.

The instant portion of the invention, as claimed, falls under the "germ of an idea" concept defined by the CAFC. The court has stated that "patent protection is granted in return for an enabling disclosure, not for vague intimations of general ideas that may or may not be workable". The court continues to say that "tossing out the mere germ of an idea does not constitute an enabling disclosure" and that "the specification, not knowledge in the art, that must supply the novel aspects of an invention in order to constitute adequate enablement". (See Genentech Inc v. Novo NordiskA/S 42 USPQ2d 1001, at 1005). The claimed methods of

enhancing rAAV transduction comprising contacting a mammalian cell with an enormous genus of structurally and functionally diverse compositions so as to affect a broad genus of distinctly different and mutually exclusive cell biological processes constitute such a "germ of an idea".

The courts have stated that reasonable correlation must exist between scope of exclusive right to patent application and scope of enablement set forth in patent application. 27 USPQ2d 1662 *Exparte Maizel*. In the instant case, in view of the lack of guidance, working examples, breadth of the claims, the level of skill in the art and state of the art at the time of the claimed invention was made, it would have required undue experimentation to make and/or use the invention as claimed.

In conclusion, the specification fails to provide any guidance as to how an artisan would have dealt with the art-recognized limitations of the claimed method commensurate with the scope of the claimed invention and therefore, limiting the claimed invention to an *in vitro* method to enhance recombinant adeno-associated (rAAV) transduction of a mammalian cell comprising contacting the mammalian cell with at least one rAAV and two different agents that each enhance intracellular rAAV transduction of a mammalian cell in an amount effective to more than additively enhance rAAV transduction, wherein the first agent is doxorubicin and the second agent is the tripeptide aldehyde N-acetyl-L-leucyl-L-leucyl-norleucinal (LLnL) or N-carbobenzoxyl- L-leucinyl-L-leucinyl-L-leucinal (Z-LLL) that inhibits proteosome proteolytic activity, is proper.

Response to Arguments

Applicant argues that preparation of a wide variety of rAAV is within the skill of the art

Applicant's argument(s) has been fully considered, but is not persuasive. The examiner has not set forth the position that the preparation of a wide variety of rAAV is outside the skill of the art. The Examiner has considered Douar (2001) discussing *in vitro* rAAV infection and intracellular trafficking, and the Muzyczka (1992) abstract broadly reviewing AAV vectors.

The examiner is unable to find a copy of Arruda (2004), Denby (2005), Harding (2006), Jennings (2005) or Sarkar (2004) in the response filed May 4, 2009 or in any of the IDS'es, as indicated in the remarks.

Applicant argues that the claims recite that each agent enhances AAV transduction and so excludes agents that do not enhance AAV transduction of particular cell types (see M.P.E.P. §2164.08(b)). Further, the specification discloses the infection of HeLa cells, IB3 cells, A549 cells, ferret fibroblasts, mouse lung, trachea and bronchi, and airway epithelia with rAAV.

Applicant's argument(s) has been fully considered, but is not persuasive. As a first matter, the specification clearly evidences that enhanced transduction is not achieved by the same combination of agents with all cell types. The specification provides evidence for only the combination of doxorubicin and the tripeptide aldehyde Z-LLL or LLnL. See also Declaration, Table 1. As a second matter, limitations and examples in the specification do not generally limit what is covered by the claims. MPEP §2164.08 The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984) M.P.E.P. §2164.08(b)). Claims reading on significant numbers of inoperative embodiments would render claims nonenabled when the specification does not clearly identify the operative embodiments and undue experimentation is involved in determining those that are operative. *Atlas Powder Co. v. E.I. duPont de Nemours & Co.,* 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984); *In re Cook*, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971). In the instant case, the specification discloses only two operative embodiments, doxorubicin in combination with LLnL or Z-LLL, and fails to provide the necessary guidance to inform the artisan of multitude of embodiments of two or more agent combinations that are inoperative, and it would require undue experimentation for the artisan to determine for themselves which embodiment(s) is operable and which is not. The claims require undue experimentation because the artisans must determine for themselves:

i) the *in vivo* dosing and dosage regimen of an enormous genus of two or more agent combinations to administer to a host subject with no predictable expectation of success to find a combination that achieves a more than additive effect in enhancing rAAV transduction,

ii) the *in vitro* and/or *ex vivo* dosing of an enormous genus of two or more agent combinations to an enormous genus of mammalian cell types with no predictable expectation of

success to find a combination that achieves a more than additive effect in enhancing rAAV transduction,

iii) the appropriate and unpredictable timepoint after the two or more agents are administered from which one is to assay for the more than additive effect *in vivo* as per a given tissue, e.g. at six weeks, but not two weeks, three weeks, etc…, in heart, lung, kidney, brain, etc..,

iii) the *in vivo* dosing and dosage regimen for each particular cell type, e.g. epithelial, endothelial, hematopoietic, neural, etc…, because the results achieved with a first cell type are not predictably extrapolated to the results one may expect to achieve with a second cell type, as evidenced by Applicant's own work (Declaration, Table 1),

iv) assaying each rAAV serotypes 1-11, and permutations thereof, for each two or more agent combinations for each cell type,

v) the *in vivo* dosing and dosage regimen to avoid cumulative systemic toxicity of the two or more agent(s), and

vi) whether or not the results of each two or more agent combinations that achieves a more than additive effect *in vitro* can be predictably extrapolated to usage *in vivo*.

Applicant argues that *in vivo* use of rAAV, chemotherapeutics, lipid lowering agents, antibiotics, tannic acid, see Duan (2000), Wu (2008), Chen (2008) and Hsu (2008).

Applicant's argument(s) has been fully considered, but is not persuasive. The Examiner has not argued that each agent and each rAAV has never been used *in vivo* before the instantly claimed method. The issue is that the use of an agent individually for a different purpose, i.e. chemotherapy, does not immediately inform the artisan how to use the ***combination*** of such two or more agents for a different purpose, to wit, enhancing rAAV transduction. Applicants declare (Englehardt and Yan Decl., filed May 6, 2009; see discussion below) that in the absence of data, it is not predictable what combinations of agents have any effect, let alone have an additive or synergistic effect on rAAV transduction (¶8).

Duan (2000) teaches the use of Z-LLL alone to enhance rAAV transduction *in vivo* (Figure 11), but is silent regarding the administration of **a combination of two or more agents**

to enhance rAAV transduction, and thus is not commensurate in scope with the instantly claimed method.

Wu (2008) abstract teaches enhancing rAAV transduction with an adenovirus vector, but is silent regarding the administration of **a combination of two or more agents** to enhance rAAV transduction, and thus is not commensurate in scope with the instantly claimed method.

Chen (2008), Hsu (2008) abstracts teach *in vivo* delivery of rAAV, but are silent regarding the administration of **a combination of two or more agents** to enhance rAAV transduction, and thus are not commensurate in scope with the instantly claimed method.

Applicant argues that serotypes other than AAV-2 may be employed to achieve organ-specific tropisms, as taught by Arruda et al (2004).

Applicant's argument(s) has been fully considered, but is not persuasive. As a first matter, the examiner is unable to find a copy of Arruda (2004) in the response filed May 4, 2009 or in any of the IDS'es, as indicated in the remarks.

As a second matter, the instant specification discloses only the use of rAAV2 (predominantly) and rAAV5 in the working examples. Applicant's own work clearly evidences that no predictable correlation is found between rAAV serotypes (rAAV-2 or rAAV-5) and enhanced transduction of a particular mammalian cell type (IB3, A549, HeLa) (¶11, Tables 1 and 2). That is to say, a synergistic effect between a first and second agent in a first cell type with a first rAAV serotype cannot be predictably extrapolated to a second cell type and a second rAAV serotype. The artisans must discover for themselves which rAAV should/must be used for which organ-specific tropism.

As a third matter, limitations and examples in the specification do not generally limit what is covered by the claims. MPEP §2164.08 The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984) M.P.E.P. §2164.08(b)). Claims reading on significant numbers of inoperative embodiments would render claims nonenabled when the specification does not clearly identify the operative embodiments and undue experimentation is involved in determining those that are

operative. *Atlas Powder Co. v. E.I. duPont de Nemours & Co.,* 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984); *In re Cook*, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971). In the instant case, the specification discloses only two operative embodiments, rAAV2 and rAAV5, and fails to provide the necessary guidance to inform the artisan those rAAV serotypes whose transduction efficiency would not be enhanced [inoperative] more than additively by a combination of two or more agents, and it would require undue experimentation for the artisan to determine for themselves which rAAV serotype embodiment(s) is operable in which two or more agent combinations and which is not. The claims require undue experimentation because the artisans must determine for themselves:

i) the *in vivo* dosing and dosage regimen of an enormous genus of two or more agent combinations to administer to a host subject with no predictable expectation of success to find a combination that achieves a more than additive effect in enhancing rAAV transduction,

ii) the *in vitro* and/or *ex vivo* dosing of an enormous genus of two or more agent combinations to an enormous genus of mammalian cell types with no predictable expectation of success to find a combination that achieves a more than additive effect in enhancing rAAV transduction,

iii) the appropriate and unpredictable timepoint after the two or more agents are administered from which one is to assay for the more than additive effect *in vivo* as per a given tissue, e.g. at six weeks, but not two weeks, three weeks, etc…, in heart, lung, kidney, brain, etc..,

iv) assaying each rAAV serotypes 1-11, and permutations thereof, for each two or more agent combinations for each cell type,

v) the *in vivo* dosing and dosage regimen for each particular cell type, e.g. epithelial, endothelial, hematopoietic, neural, etc…, because the results achieved with a first cell type are not predictably extrapolated to the results one may expect to achieve with a second cell type, as evidenced by Applicant's own work (Declaration, Tables 1 and 2),

vi) the *in vivo* dosing and dosage regimen to avoid cumulative systemic toxicity of the two or more agent(s), and

vii) whether or not the results of each two or more agent combinations that achieves a more than additive effect *in vitro* can be predictably extrapolated to usage *in vivo*.

Applicant argues that the specification provides adequate direction and guidance (pg 51-52; pg 86) and working examples.

Applicant's argument(s) has been fully considered, but is not persuasive. As discussed in the above rejection, the guidelines provided by the specification are general at best, and specific only for doxorubicin in combination with LLnL or Z-LLL. Applicant states that because of its cytotoxicity, the use of doxorubicin in a clinical setting is counter indicated due to its cumulative systemic toxicity. In addition, the use of a toxic agent, such as doxorubicin, may compromise cell viability, thereby defeating the goal of enhanced transduction of cells with rAAV encoding a therapeutic gene product. Given that doxorubicin binds DNA, it might be expected to inhibit rAAV transduction, as rAAV is present as a double-stranded DNA molecule in the nucleus (¶7). However, the specification fails to disclose how to overcome this cumulative cytotoxicity so as to use rAAV vectors encoding a therapeutic gene, i.e. CFTR, for the treatment of chronic diseases such as cystic fibrosis, which is a real-world embodiment of the instantly claimed invention.

Applicant argues that practitioners in the art related to the present application would be well-equipped to prepare and/or screen combinations of agents falling within the scope of the claims to identify those agents that at least additively enhance AAV transduction, citing *Hybritech Inc. v. Monoclonal Antibodies Inc.*, 23 1 U.S.P.Q. 8 1, 84 (Fed. Cir. 1986) (evidence that screening methods used to identify characteristics [of monoclonal antibodies] were available to art convincing of enablement).

Applicant's argument(s) has been fully considered, but is not persuasive. *Hybritech Inc. v. Monoclonal Antibodies Inc.*, 23 is not on point per the instant claims because those of ordinary skill in the art recognize that it is both routine and predictable that a given antigen will cause an antibody response, thereby allowing the artisan to screen for and identify the antibody that binds said antigen. Thus, the artisan can predictably expect to achieve the desired result. However, the instant claims are directed to a combination of two or more agents to achieve an unpredictable effect, to wit, to more than additively enhance, *in vitro* and *in vivo*, rAAV transduction of a multitude of rAAV serotypes having distinctly different tropisms for distinctly different cell

types. Applicants declare (Englehardt and Yan Decl., filed May 6, 2009; see discussion below) that in the absence of data, it is not predictable what combinations of agents have any effect, let alone have an additive or synergistic effect on rAAV transduction (¶8). Compounds with similar mechanisms of action may, under some conditions, yield a positive, but not synergistic, effect (¶8). No predictable correlation is found between rAAV serotypes (rAAV-2 or rAAV-5) and enhanced transduction of a particular mammalian cell type (IB3, A549, HeLa) (¶11, Table 1). That is to say, a synergistic effect between a first and second agent in a first cell type with a first rAAV serotype cannot be predictably extrapolated to a second cell type and a second rAAV serotype. The effect of a first double agent combination (Dox and LLnL) does not predictably extrapolate to a second double agent combination (Velcade® and LLnL; Velcade® and Dox) (Table 1). It is not predictable what combinations of agents have any effect, let alone a positive or synergistic effect, on AAV transduction (¶13). Thus, the preponderance of evidence of record clearly establishes that it is neither routine nor predictable for the artisan expect a given combination of two or more agents to yield a more than additively enhancement in rAAV transduction, either *in vitro* or *in vivo*, regardless of rAAV serotype and regardless of cell type.

Applicant argues that the fact that a given claim may encompass a variety of agents, mammalian cells and rAAVs is not dispositive of the enablement issue, particularly in an art area in which the level of skill is very high and in which screening of large numbers of compounds has been standard practice for at least ten years (*Ex parte Forman*, 230 U.S.P.Q.2d 456 (Bd. App. 1986

Applicant's argument(s) has been fully considered, but is not persuasive. *Ex parte Forman* is not on point per the instant claims because *Forman* is drawn to the screening and isolating of bacterial strains to yield a useful vaccine. However, the art pertaining to the instant claims is nascent, three years prior (not ten years as Applicant argues) to Applicant's provisional specification (Duan et al, 2000). Furthermore, while Duan et al teach the small-scale *in vitro* assay for agents (seven compounds) that enhance rAAV2 transduction and a combination of agents (one combination; Figure 5) that more than additively enhances rAAV2 transduction, the prior art is silent regarding large-scale screening for a combination of two or more agents to

enhance, *in vitro* and/or *in vivo*, a multitude of distinctly different rAAV serotypes having specific tropism for a multitude of distinctly different cell types.

The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984) M.P.E.P. §2164.08(b)). Claims reading on significant numbers of inoperative embodiments would render claims nonenabled when the specification does not clearly identify the operative embodiments and undue experimentation is involved in determining those that are operative. *Atlas Powder Co. v. E.I. duPont de Nemours & Co.,* 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984); *In re Cook*, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971). In the instant case, the specification discloses only two operative embodiments, rAAV2 and rAAV5, and fails to provide the necessary guidance to inform the artisan those rAAV serotypes whose transduction efficiency would not be enhanced [inoperative] more than additively by a combination of two or more agents, and it would require undue experimentation for the artisan to determine for themselves which rAAV serotype embodiment(s) is operable in which two or more agent combinations and which is not. The claims require undue experimentation because the artisans must determine for themselves:

i) the *in vivo* dosing and dosage regimen of an enormous genus of two or more agent combinations to administer to a host subject with no predictable expectation of success to find a combination that achieves a more than additive effect in enhancing rAAV transduction,

ii) the *in vitro* and/or *ex vivo* dosing of an enormous genus of two or more agent combinations to an enormous genus of mammalian cell types with no predictable expectation of success to find a combination that achieves a more than additive effect in enhancing rAAV transduction,

iii) the appropriate and unpredictable timepoint after the two or more agents are administered from which one is to assay for the more than additive effect *in vivo* as per a given tissue, e.g. at six weeks, but not two weeks, three weeks, etc…, in heart, lung, kidney, brain, etc..,

iv) assaying each rAAV serotypes 1-11, and permutations thereof, for each two or more agent combinations for each cell type,

v) the *in vivo* dosing and dosage regimen for each particular cell type, e.g. epithelial, endothelial, hematopoietic, neural, etc…, because the results achieved with a first cell type are not predictably extrapolated to the results one may expect to achieve with a second cell type, as evidenced by Applicant's own work (Declaration, Tables 1 and 2),

vi) the *in vivo* dosing and dosage regimen to avoid cumulative systemic toxicity of the two or more agent(s), and

vii) whether or not the results of each two or more agent combinations that achieves a more than additive effect *in vitro* can be predictably extrapolated to usage *in vivo*.

Applicant argues that evidence that screening numerous compounds to detect the effect of the compound on virus infection or replication is within the skill of the art was provided in the abstracts for Cheng et al. (2004)) and Dhanak et al. (2002) who evidence is that it is within the skill of the art to screen a large number of compounds to identify ones with desirable activity. Thus, a library of anthracyclines or proteosome inhibitors, or combinations thereof, may be readily screened for whether or not those agents alone, or in combination additively or synergistically, enhance rAAV transduction. Such a screen does not require knowledge of the underlying cellular process that is altered by the agent(s) It is certainly within the skill of the art in view of Applicant's specification to test other agents, for instance, other functionally or structurally related agents, to determine whether they alone, or in combination with a different agent, enhance AAV transduction

Applicant's argument(s) has been fully considered, but is not persuasive. Cheng (2004) abstract teaches screening for HIV protease inhibitors, but is silent regarding the administration of **a combination of two or more agents** to enhance rAAV transduction, and thus is not commensurate in scope with the instantly claimed method. Dhanak (2002) abstract teaches screening for a compound that inhibits RNA-dependent RNA polymerase, but is silent regarding the administration of **a combination of two or more agents** to enhance rAAV transduction, and thus is not commensurate in scope with the instantly claimed method. As discussed above, the technology in the field of endeavor of the instant claims is nascent and unpredictable. Finding the

desired combination of two or more agents to achieve the desired effect neither routine nor predictable, and thus the full scope of the instant claims requires undue burden for the artisan to determine for themselves which embodiment is operable amongst those embodiments are inoperable.

Applicant argues that the art worker in possession of Applicant's specification would be apprised that the use of anthracyclines or epoxomicin or simvastatin in combination with tripeptidyl aldehydes may enhance rAAV transduction, because similar agents likely have similar activities. It is Applicant's position that determining which combinations of those agents that enhance rAAV transduction is not undue experimentation in an art where the level of skill is high. *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988).

Applicant's argument(s) has been fully considered, but is not persuasive. Applicant's argued position that similar agents likely have similar activities is contradictory to Applicant's declaration (Englehardt and Yan Decl., filed May 6, 2009; see discussion below) that in the absence of data, it is not predictable what combinations of agents have any effect, let alone have an additive or synergistic effect on rAAV transduction (¶8). Compounds with similar mechanisms of action may, under some conditions, yield a positive, but not synergistic, effect (¶8). No predictable correlation is found between rAAV serotypes (rAAV-2 or rAAV-5) and enhanced transduction of a particular mammalian cell type (IB3, A549, HeLa) (¶11, Table 1). That is to say, a synergistic effect between a first and second agent in a first cell type with a first rAAV serotype cannot be predictably extrapolated to a second cell type and a second rAAV serotype. The effect of a first double agent combination (Dox and LLnL) does not predictably extrapolate to a second double agent combination (Velcade® and LLnL; Velcade® and Dox) (Table 1). It is not predictable what combinations of agents have any effect, let alone a positive or synergistic effect, on AAV transduction (¶13). As discussed above, the technology in the field of endeavor of the instant claims is nascent and unpredictable. Finding the desired combination of two or more agents to achieve the desired effect neither routine nor predictable, and thus the full scope of the instant claims requires undue burden for the artisan to determine for themselves which embodiment is operable amongst those embodiments are inoperable.

Claim Rejections - 35 USC § 103

5. **The prior rejection of Claims 1-2, 5-7, 9-23, 43-44, 48-50 and 64 under 35 U.S.C. 103(a)** as being unpatentable over Duan et al in view of Kiyomiya et al, Maitra et al and Englehardt **is withdrawn**.

The Englehardt and Yan Declaration under 37 CFR 1.132 filed May 6, 2009 is sufficient to overcome the rejection of Claims 1-2, 5-7, 9-23, 43-44, 48-50 and 64 based upon Duan et al in view of Kiyomiya et al, Maitra et al and Englehardt as set forth in the last Office action. Applicants declare that in the absence of data, it is not predictable what combinations of agents have any effect, let alone have an additive or synergistic effect on rAAV transduction (¶8). Compounds with similar mechanisms of action may, under some conditions, yield a positive, but not synergistic, effect (¶8). No predictable correlation is found between rAAV serotypes (rAAV-2 or rAAV-5) and the mammalian cell type (IB3, A549, HeLa) (¶11, Table 1). That is to say, a synergistic effect between a first and second agent in a first cell type with a first rAAV serotype cannot be predictably extrapolated to a second cell type and a second rAAV serotype. Furthermore, the effect of a first double agent combination (Dox and LLnL) does not predictably extrapolate to a second double agent combination (Velcade and LLnL; Velcade and Dox) (Table 1). It is not predictable what combinations of agents have any effect, let alone a positive or synergistic effect, on AAV transduction (¶13).

The Englehardt and Yan Declaration under 37 CFR 1.132 filed May 4, 2009 is insufficient to overcome the rejection of Claims 1-2, 5-7, 9-23, 43-44, 48-50 and 64 based upon Duan et al in view of Kiyomiya et al, Maitra et al and Englehardt as set forth in the last Office action because: the declaration is unsigned.

6. **The prior rejection of Claim 62 under 35 U.S.C. 103(a)** as being unpatentable over Duan et al in view of Kiyomiya et al, Maitra et al and Englehardt, as applied to claims 1-2, 4-7, 9-23, 43-44, 46, 48-50, 61 and 63-64 above, and in further view of Voinea et al **is withdrawn** for reasons discussed above.

7. **The prior rejection of Claim 24 under 35 U.S.C. 103(a)** as being unpatentable over Duan et al in view of Kiyomiya et al, Maitra et al and Englehardt, as applied to claims 1-2, 4-7, 9-23, 43-44, 46, 48-50 and 61-64 above, and in further view of Hirsch et al **is withdrawn** for reasons discussed above.

Conclusion

8. No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Kevin K. Hill whose telephone number is 571-272-8036. The Examiner can normally be reached on Monday through Friday, between 9:00am-6:00pm EST.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Joseph T. Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Kevin K. Hill/
Examiner, Art Unit 1633

/Anne Marie S. Wehbé/
Primary Examiner, A.U. 1633